

Sinsheimer

CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

how much cells.

~90g.

DIVISION OF BIOLOGY

May 22, 1963

VIA AIR MAIL

Professor Paul Berg
Biochemistry Department
Stanford University Medical School
Palo Alto, California

Dear Paul:

With regard to publication we will not be presenting any data at CSH, but this should not inhibit you at all. As you point out we can still submit two papers together to JMB.

With respect to the T_m - our figure is $77.5 - 78^\circ$ in 0.01M sodium citrate pH 7.0 plus 0.02 M NaCl. This is 0.05 M Na^+ which I gather from your graph you would say gave a T_m of 75° . The general shape of our melting curve is similar to yours with perhaps a little sharper bend on the low temperature side.

Our experiment on the replication of the hybrid was done by incubating H^3 -hybrid plus enzyme plus C^{14} -tri- PO_4 and examining the product (approximately 1.7 x as much RNA was made as was in the original hybrid) in a sucrose gradient. The hybrid which has an S about 13 now contained both C^{14} and H^3 (molar ratio $C^{14}/H^3 \sim 1.6$). Other counts were in free RNA (S ~ 5-10) and C^{14}/H^3 molar ratio ~ 3. This seemed like a mixture of conservative and non-conservative replication (with conservative favored) although as Mike pointed out we could partially explain this by assuming that the enzyme, present in limiting amount, selects substrate at random so that after a few C^{14} hybrids have been made, they could be used - i.e. we could not talk about the replication of individual molecules. Your experiment with 5 BUTP and C^{14} CTP is a better one, but would require careful density determinations.

We have tried to approach this by using saturating amounts of enzyme so that one might assume all hybrid primer molecules were engaged. However this got us into another difficulty - our enzyme preparations do have a small activity in the absence of added primer. With high enzyme concentration and low primer concentration this seriously confuses the result.

At present we are trying to determine the result kinetically with especial emphasis on low levels of incorporation. I will let you know how this comes out.

Professor Berg

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reasonable May 22, 1963

We have recently been studying the incorporation of deoxyATP into what appears to be a ϕ X-DNA-RNA hybrid. You state in your paper with Mike that deoxynucleotide-triphosphates do not work, but at least with ϕ X DNA, and one deoxytriphosphate this seems to go fairly well. The product has a reasonable density. My only concern is that the C^{14} -dATP we are using might have some rATP in it - however reconstruction experiments suggest it would have to be as much as 5% which seems unlikely. We are doing the appropriate degradation experiments on the product which should provide a definite answer. Such products should be of considerable value in sequence studies.

With best regards,

Bob

RLS:e

R. L. Sinsheimer
Professor of Biophysics

P.S. I hesitate to ask but if I wonder if a return for the ϕ X DNA we would obtain either some E. Coli cells or some polymerase from you. Our problem is that our Bogen has been out of order for three months now. It has been "fixed" twice and each time has not emerged a full run - and I begin to despair when it will be functional. In all meantime we are now virtually out of enzyme.